Growing E. coli for visibility test

Aim: Experiencing and trying different bacterial concentrations for the purpose of choosing the right one for the chip.

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- **For each** strain of bacteria (5), prepare sterile:
  - Small, autoclaved, 250ml erlenmeyers.
  - 25 ml of sterile LB (maximum 1/10 of the erlenmeyers' volume).
  - Antibiotic, matching to the strand resistance.

    2 CM & 3 AMP, at Concentration of 1/1000 from stock.
- Sterile inoculating loop.
- adhesive tape.
- Cuvette.
- Test tubes.
- Aluminum sheet.

Protocol:

Day 1:

1. For each color of bacteria, prepare an erlenmeyers as mentioned.
2. Use a sterile inoculating loop to pick up a colony from a plate/ starter/ glycerol stock.
3. Place the loop into the LB and swirl to completely transfer the bacteria.
4. Cover the erlenmeyers using aluminum sheet, allowing air to get in and out, and fix using an adhesive tape.
5. Make sure to mark the erlenmeyers: Name, date, strain, medium.
6. Place in a $37°_{C}$ shaker overnight.

Day two:

1. Set the spectrophotometer blank on sterile LB.

2. From each erlenmeyer, transfer 1ml to a cuvette and read the result.
3. If OD>0.6, dilute the 1ml sample to OD<0.6.

   Write down the bacterial OD in the flask.
4. From each flask, prepare a series of dilution.
5. Masseur each dilation OD's, check if the color is visible on the chip and take a picture under proper lighting.

If no color visible:

a. Centrifuge the bacteria for 5 min at 5000 RPM.
b. Resuspend bacteria in a smaller LB volume (write down the concentration factor).
c. Proceed to the 4th step of day 2.

Notes:

- The bacteria are being checked at their stationary phase.